$$R_u = \frac{\text{Area of spectinomycin sample peak (at a retention time equal to that observed for the spectinomycin standard)}}{\text{Area of internal standard peak}}$$

$$R_s = \frac{\text{Area of the spectinomycin standard peak}}{\text{Area of internal standard peak}}$$

W_s=Weight of the spectinomycin working standard in milligrams;
D=Dilution of the spectinomycin dose;
f=Potency of the spectinomycin working standard in milligrams of spectinomycin per milligram.

(2) *Microbiological activity* (*microbiological turbidimetric assay*). Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 30.0 micrograms of spectinomycin per milliliter (estimated).

(3) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Pyrogens*. Proceed as directed in §436.32(a) of this chapter, using a solution containing 50 milligrams of spectinomycin base per milliliter.

(5) [Reserved]

(6) *Depressor substances*. Proceed as directed in §436.35 of this chapter.

(7) *Moisture*. Proceed as directed in §436.201 of this chapter.

(8) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter, except, if it is packaged for dispensing, use the suspension obtained after reconstituting the drug as directed in the labeling.

(9) *Identity test*. Proceed as directed in §436.211 of this chapter, using the method described in paragraph (b)(2) of that section.

(10) *Residue on ignition*. Proceed as directed in §436.207 of this chapter, using the method described in paragraph (b) of that section.

(11) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19921, May 13, 1985]

§455.82a Sterile sulbactam sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile sulbactam sodium is sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4 dioxide. It is so purified and dried that:

(i) Its sulbactam potency is not less than 886 micrograms and not more than 941 micrograms per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1 percent.

(v) It is crystalline.

(vi) It passes the identity test for sulbactam sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 30 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 230 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silica, a flow rate of 2.0 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *1.0M Phosphoric acid*. Prepare by dissolving 67.5 milliliters of reagent grade phosphoric acid (85 percent) in distilled water and dilute to 1 liter.

(B) *0.005M Tetrabutylammonim hydroxide.* Dilute 6.6 milliliters of tetrabutylammonium hydroxide (40 percent) to 1,800 milliliters with distilled water. Adjust the pH to 5.0 with 1.0*M* phosphoric acid and dilute with distilled water to 2 liters.

(C) *Mobile phase.* Mix 350 milliliters of acetonitrile with 1,650 milliliters of 0.005*M* tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chromatographic pumping system. (Slight adjustments in pH and/or acetonitrile content may be made to achieve the system suitability parameters defined in paragraph (b)(1)(iii) of this section.)

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Dissolve an accurately weighed portion of sulbactam working standard in sufficient mobile phase to give a stock solution of a known concentration containing about 1 milligram of sulbactam per milliliter.

(B) *Sample solution.* Dissolve an accurately weighed portion of the sample in sufficient mobile phase to give a stock solution containing 1 milligram of sulbactam per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5 at 10 percent of peak height in lieu of 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory for sulbactam if it is greater than 3,500 theoretical plates for a 30-centimeter column.

(C) *Resolution.* The resolution (*R*) between the peaks for sulbactam and penicillanic acid is satisfactory if it is not less than 3.8.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations.* Calculate the micrograms of sulbactam per milligram of sample as follows:

$$\text{Micrograms of sulbactam per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the sulbactam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the sulbactam peak in the chromatogram of the sulbactam working standard;

P_s=Sulbactam activity in the sulbactam working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 20 milligrams of sulbactam per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the sulbactam working standard.

[52 FR 42290, Nov. 4, 1987; 52 FR 45281, Nov. 25, 1987, as amended at 54 FR 47205, Nov. 13, 1989; 55 FR 11585, Mar. 29, 1990]

§ 455.85 Vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]